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Review

The toolbox of *Trichoderma* spp. in the biocontrol of *Botrytis cinerea* disease

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SUMMARY

Botrytis cinerea is a necrotrophic fungal pathogen causing disease in many plant species, leading to economically important crop losses. So far, fungicides have been widely used to control this pathogen. However, in addition to their detrimental effects on the environment and potential risks for human health, increasing fungicide resistance has been observed in the *B. cinerea* population. Biological control, that is the application of microbial organisms to reduce disease, has gained importance as an alternative or complementary approach to fungicides. In this respect, the genus *Trichoderma* constitutes a promising pool of organisms with potential for *B. cinerea* control. In the first part of this article, we review the specific mechanisms involved in the direct interaction between the two fungi, including mycoparasitism, the production of antimicrobial compounds and enzymes (collectively called antagonism), and competition for nutrients and space. In addition, biocontrol has also been observed when *Trichoderma* is physically separated from the pathogen, thus implying an indirect systemic plant defence response. Therefore, in the second part, we describe the consecutive steps leading to induced systemic resistance (ISR), starting with the initial *Trichoderma*–plant interaction and followed by the activation of downstream signal transduction pathways and, ultimately, the defence response resulting in ISR (ISR-prime phase). Finally, we discuss the ISR-boost phase, representing the effect of ISR priming by *Trichoderma* spp. on plant responses after additional challenge with *B. cinerea*.

Keywords: biological control, grey mould, induced systemic resistance, transcriptomics.

INTRODUCTION

Plants are surrounded by a diverse range of organisms in their environment, including bacteria, fungi, oomycetes, nematodes, insects and viruses. Although some of these organisms may have a negative impact on the plant, others may exert beneficial effects by enhancing the general fitness of the plant and/or by suppressing plant disease. Research on such biocontrol organisms (BCOs) has intensified during recent decades and their importance has increased as a part of integrated management practices to reduce chemical pesticide use (Glare *et al*., 2012). Early biocontrol research mainly focused on BCOs exerting a protective effect by direct interaction with pathogens. Interestingly, some BCOs were later found to be effective against pathogens through indirect interactions mediated by the plant, as the BCOs and pathogen were spatially separated. In this case, the BCO causes a reprogramming of the plant's gene expression, leading to induced systemic resistance (ISR; Shoresh *et al*., 2010; Van Wees *et al*., 2008).

The genus *Trichoderma* comprises a great number of fungal strains with BCO capacity. They have adapted to diverse environmental conditions and are often the most frequently isolated fungi from soil (Harman *et al*., 2004). They are prolific producers of extracellular proteins, including enzymes that degrade cellulose and chitin, which are widely used in industrial applications (Nagy *et al*., 2007). Moreover, their capacity to reduce plant disease has been studied intensively, with both direct and indirect effects on plant pathogens (Lorito *et al*., 2010). Their high reproductive capacity, ability to survive under unfavourable conditions and high nutrient utilization efficiency contribute to their success as BCOs (Benitez *et al*., 2004). Many *Trichoderma* strains are able to colonize plant roots of both dicots and monocots, and trigger ISR, which is effective against a wide range of pathogens. Given that *Trichoderma* spp. are also capable of living freely in soil, they are considered to be opportunistic plant symbionts (Harman *et al*., 2004).

The application of *Trichoderma* spp. against one of the economically most important pathogens, the necrotrophic fungus *B. cinerea*, offers interesting perspectives. This pathogen causes disease in more than 200 plant species, including numerous crops, attacking organs such as leaves, stems, fruits and flowers, both pre- and post-harvest (Elad *et al*., 2004). The occurrence of soft rot, accompanied by collapse and water soaking of parenchyma tissues, and the subsequent rapid appearance of grey conidial masses,

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represent the most typical symptoms on leaves and fruits, referred to as grey mould (Williamson *et al*., 2007). The fungus has a predominant necrotrophic lifestyle, implying that it kills plant host cells by producing diverse phytotoxic compounds and cell wall-degrading enzymes (CWDEs), after which it extracts nutrients from the dead cells (Lazniewska *et al*., 2010). *Botrytis cinerea* is difficult to control because of its different attacking modes, survival under unfavourable conditions for extended periods as sclerotia in crop debris and development of fungicide-resistant strains (Williamson *et al*., 2007). The global expenses for *B. cinerea* control easily surmount €1 billion per annum; however, the impact of product and quality loss occurring despite all control measures is expected to be much higher (Dean *et al*., 2012). In addition, potentially harmful consequences associated with the use of fungicides for both humans and the environment are an incentive to develop alternative and/or complementary management approaches. This has led to the evaluation of potential BCOs capable of substantial disease suppression in a commercial context and within integrated crop management systems (Elad *et al*., 2004). One of the key microbial genera to show great potential for the control of *B. cinerea* disease is the above-mentioned genus *Trichoderma* (some recent reports include Martínez-Medina *et al*., 2013; Mathys *et al*., 2012; Tucci *et al*., 2011). This review elaborates on the different modes of action in the control of *B. cinerea* by *Trichoderma* spp., thereby discussing both direct and indirect interactions.

DIRECT INTERACTIONS

Several *Trichoderma* strains have been reported to inhibit *B. cinerea* directly, in both soil and on plant surfaces. The specific mechanisms involved in direct interactions comprise mycoparasitism, antibiosis and competition. The different modes of action are described here, specifically for the *Trichoderma* spp.–*B. cinerea* interaction.

Mycoparasitism

Mycoparasitism is an important biocontrol trait of the *Trichoderma* genus. A recent survey of over 1100 *Trichoderma*/*Hypocrea* strains from 75 molecularly defined species revealed that all the tested species possessed mycoparasitic potential against *B. cinerea* (Druzhinina *et al*., 2011). Mycoparasitism is a multi-step process in which physical contact between two micro-organisms is preceded by an early recognition stage. *Trichoderma* spp. are believed to constitutively secrete CWDEs at low levels in an attempt to locate potential prey. When fungal cell walls are encountered and degraded by these enzymes, oligomers are released, causing the induction of further *Trichoderma* CWDEs and directional growth towards the prey, in order to enable physical attack (Mukherjee *et al*., 2012). Evidence for this recognition comes, for example, from transcriptomic studies showing the induction of CWDE genes before actual contact with *B. cinerea* (Seidl *et al*., 2009). Once the fungi come into contact, *Trichoderma* spp. attach to the prey, coil around it and form appressoria on the host surface. During this process, *Trichoderma* spp. continue to produce CWDEs and probably also antibiotic compounds in order to further degrade their prey (Shoresh *et al*., 2010). For example, microscopic observations have revealed that *T. atroviride* LU132 grows alongside hyphae of *B. cinerea* and tightly coils around it. Although penetration was not observed after this initial interaction, *B. cinerea* hyphae rapidly collapsed and died within 4 days after inoculation (Card *et al*., 2009).

The recently published genome sequences of three *Trichoderma* spp. confirm that CWDEs play an important role in *Trichoderma* spp. biocontrol, as genes encoding the CWDEs β-1,3-glucanases were over-represented in their genomes compared with those of other related fungi (Kubicek *et al*., 2011; Mukherjee *et al*., 2013). In addition, the genomes of two highly mycoparasitic species (*T. virens* and *T. atroviride*) were enriched in secondary metabolism-related genes compared with the weakly mycoparasitic *T. reesei* (Kubicek *et al*., 2011). Yang *et al*. (2009) reported that the presence of *B. cinerea* can specifically elicit the production of CWDEs in *T. harzianum* ETS323. The authors compared the secreted protein patterns of this strain grown in the presence or absence of *B. cinerea*, and found that two endochitinases were uniquely induced in the presence of *B. cinerea*, which also led to higher β-1,3-glucanase, β-1,6-glucanase and protease activity than in the absence of the pathogen. Similarly, the expression of α-1,3-glucanase was induced in *T. asperellum* T32 in direct confrontation assays with *B. cinerea* (Sanz *et al*., 2005).

Trichoderma spp. thus seem to have the perfect enzyme arsenal to target *B. cinerea* cell walls, in which chitin and non-cellulosic β-glucan are the main skeletal or microfibrillar polysaccharides, with proteins and α-glucans as the main cementing substances binding together the different structural cell wall components into macromolecular complexes (Cantu *et al*., 2009b). In addition, substantial amounts of dark pigment, melanin, can also be present in the *B. cinerea* cell wall and extracellular matrix of *B. cinerea* germlings and sclerotia (Cantu *et al*., 2009b). Melanin is formed by oxidative polymerization of various phenolic compounds and, as such, can be degraded by phenol-oxidases, such as laccase (Giardina *et al*., 2010). Catalano *et al*. (2011) found that the laccase gene *Lcc1* was specifically highly expressed in *T. virens* during the early phases of contact with *B. cinerea* sclerotia, and that the *T. virens* deletion mutant Δ*lcc1* showed a significantly decreased ability to decay these sclerotia.

Antibiosis

Trichoderma spp. also produce a plethora of secondary metabolites with inhibitory activity against a diverse range of

microorganisms. The production of secondary metabolites in *Trichoderma* spp. can be strain dependent, and includes both volatile and non-volatile antimicrobial substances belonging to a variety of chemical compound classes (Reino *et al*., 2008). These include low-molecular-weight non-polar compounds, such as pyrones, butenolides, azaphylones, anthraquinones, trichothecenes, terpenoids and steroids, as well as non-ribosomal peptides, such as siderophores and peptaibols (Mukherjee *et al*., 2012).

One of the best-studied secondary metabolites from a biocontrol perspective is the 'coconut aroma' volatile pyrone 6-pentyl-2*H*-pyran-2-one, commonly produced by *Trichoderma* spp. (Vinale *et al*., 2008). Its antifungal activity against *B. cinerea* has been demonstrated *in vitro* (Pezet *et al*., 1999) and *in vivo*, e.g. on naturally infected kiwi fruit (Poole *et al*., 1998). Other secondary metabolites with proven *in vivo* antimicrobial activity against *B. cinerea* include the butenolides T39butenolide and harzianolide, secreted by *T. harzianum* T39, as well as T22azaphilone and an *N*-heterocyclic compound (harzianopyridone), secreted by *T. harzianum* T22 (Vinale *et al*., 2009). Another important group of compounds with antifungal activity produced by *Trichoderma* spp. are the anthraquinones. Liu *et al*. (2009) purified six compounds of this group from *T. harzianum* A6, all reducing *B. cinerea* disease incidence *in vitro*. Malmierca *et al*. (2012) showed that mutants defective in the formation of trichothecene mycotoxins (e.g. harzianum A) had reduced antifungal activity against *B. cinerea*. A novel hydroxyl-lactone, trivially named cerinolactone, was recently isolated from culture filtrates of *T. cerinum* and possesses *in vitro* antifungal activity against *B. cinerea* (Vinale *et al*., 2012).

Peptaibols represent another structurally different group of metabolites exerting antifungal activity. They constitute a class of linear short-chain-length (≤25 residues) non-ribosomal peptides of fungal origin. Their amphipathic nature allows them to self-associate into oligomeric ion channels which span the width of lipid bilayer membranes, through which the leakage of cytoplasmic material can occur, leading to cell death (Chugh and Wallace, 2001). Schirmbock *et al*. (1994) showed that the synthesis of *T. harzianum* peptaibols trichorzianine A1 and B1 was triggered by *B. cinerea* cell walls, and that they acted synergistically with chitinases and β-1,3-glucanases in inhibiting *B. cinerea* spore germination and hyphal elongation.

Enzymes with activities other than cell wall degradation have also been reported to contribute to the direct biocontrol potential of *Trichoderma* spp. against *B. cinerea*. For example, Yang *et al*. (2011) identified homodimeric L-amino acid oxidases (LAAOs) among the secreted proteins of *T. harzianum* ETS323. LAAOs are flavoenzymes that catalyse the stereospecific oxidative deamination of L-amino acid substrates to the corresponding α-ketoacids, with the release of hydrogen peroxide and ammonia (Zhang *et al*., 2003). Recently, it was shown that LAAO derived from *T. harzianum* ETS323 was able to lyse *B. cinerea* hyphae, which was accompanied by apoptosis-related events, such as DNA fragmentation, caspase pathway activation (e.g. the induction of cytochrome *c*, caspase 3 and caspase 9) and reactive oxygen species (ROS) generation (Cheng *et al*., 2012a, b).

Competition

Necrotrophic pathogens, such as *B. cinerea*, often rely on exogenous nutrients in order to germinate and grow on a plant surface before penetration (Benitez *et al*., 2004). A reduction in nutrient concentration thus generally results in reduced conidial germination and slower germ tube growth of the pathogen, thereby reducing the number of infection sites and the extent of subsequent necrosis caused by the pathogen (Nassr and Barakat, 2013). The point of entry for *B. cinerea* into the plant tissue comprises wounds, senescing host tissues or natural openings, such as stomata and lenticels, which are generally nutrient-rich areas owing to the exudation of sugars and amino acids. Therefore, by colonizing wounds or senescing tissue, *Trichoderma* spp. can compete for nutrients with *B. cinerea*, thereby preventing infection. Card *et al*. (2009) demonstrated that *T. atroviride* inhibits *B. cinerea* on strawberry leaves through different mechanisms, including competition for nutrients. Indeed, they observed a 25% reduction in the length of *B. cinerea* germ tubes present on *Trichoderma*-treated leaves relative to untreated leaves when glucose was limited. This difference was negligible when the glucose concentration was increased. In the presence of other sugars, such as sucrose and fructose, however, a significant inhibitory effect of *T. atroviride* was noticed at all tested concentrations of the sugar.

INDIRECT INTERACTIONS

In some cases, a biocontrol effect of *Trichoderma* spp. on *B. cinerea* disease has been observed, even though both fungi are physically separated from each other, indicating the involvement of a plant-mediated systemic response (De Meyer *et al*., 1998; Horst *et al*., 2005; Olson and Benson, 2007; Tucci *et al*., 2011). The consecutive steps in setting up a tripartite *Trichoderma* spp.–plant–*B. cinerea* interaction, resulting in the biocontrol of the disease, are further discussed in more detail in this section, covering the initial *Trichoderma*–plant interaction, the activation of downstream signal transduction pathways and defence responses resulting in ISR, and, finally, the effect of ISR on plant responses after *B. cinerea* challenge.

Initiation of the *Trichoderma*–plant interaction and recognition of *Trichoderma* by the plant

Some *Trichoderma* strains colonize only local sites on roots, whereas rhizosphere-competent strains colonize large root

surfaces (Harman and Shoresh, 2007). During this process, the hyphae of *Trichoderma* spp. coil around the roots, form appressoria-like structures and, finally, penetrate the root cortex, with morphological features reminiscent of those seen during mycoparasitism (Shoresh *et al*., 2010). The *Trichoderma* spp. then often grow intercellularly in the root epidermis and cortex, albeit usually limited to the first or second cell layers (Hohmann *et al*., 2012), inducing the deposition of cell wall material and the production of phenolics in surrounding cells, which limits their further ingress inside the root (Mandal and Mitra, 2007; Yedidia *et al*., 1999).

The induction of a plant-mediated ISR response starts with the recognition of *Trichoderma* by the plant, in a similar manner as for pathogens. Collectively, the general determinants of microbes that are recognized by the plant are referred to as microbe-associated molecular patterns (MAMPs) (Boller and Felix, 2009). A diversity of MAMPs of beneficial microorganisms have been shown to be implicated in the onset of ISR (Van der Ent *et al*., 2009), and this is also an active research domain for *Trichoderma* spp. in particular. In the interaction zone with the plant, *Trichoderma* spp. release many compounds that can induce ISR in plants, similar to those involved in the direct interaction with fungal pathogens (Hermosa *et al*., 2012, 2013). For example, *Trichoderma*-secreted proteins with enzymatic activity and other proteins and peptides with hydrophobin-like properties can enhance disease resistance (Djonovic *et al*., 2006; Martinez *et al*., 2001; Rotblat *et al*., 2002; Seidl *et al*., 2006). A number of reports have indicated that also peptaibols and other secondary metabolites produced by *Trichoderma* spp. can elicit plant defence responses, in addition to their direct antifungal activity (Engelberth *et al*., 2001; Vinale *et al*., 2008; Viterbo *et al*., 2007). A different class of plant defence elicitors includes oligosaccharides and low-molecular-weight compounds which are released from fungal or plant cell walls as a consequence of the activity of *Trichoderma* enzymes (Harman *et al*., 2004; Woo and Lorito, 2007; Woo *et al*., 2006).

Jones and Dangl (2006) comprehensively described the two-branched immune system involved in the plant response to pathogens as the so-called 'zig-zag model'. Interaction of a pathogen-derived MAMP with the corresponding transmembrane pattern recognition receptor (PRR) of the plant activates a primary defence response, called MAMP-triggered immunity (MTI). In a second phase, successful pathogens deploy effectors that contribute to pathogen virulence and interfere with MTI, resulting in effector-triggered susceptibility (ETS). Effector-triggered immunity (ETI), however, is based on the highly specific, direct or indirect interaction of these pathogen effectors and the products of plant resistance (*R*) genes, according to the gene-for-gene resistance theory. The *R* genes usually encode proteins of the nucleotide binding-leucine-rich repeat (NB-LRR) class, cytoplasmic proteins with a nucleotide binding site (NBS) in front of a series of leucine-rich repeats (LRRs) (Boller and Felix, 2009; Jones and Dangl, 2006; Monaghan and Zipfel, 2012). *Trichoderma* spp. may affect the plant response by increasing its basic immunity or MTI (Lorito *et al*., 2010). Indeed, using a transcriptomic approach and subsequent gene ontology (GO) analysis, we observed the induction of the MTI response in *Arabidopsis thaliana* leaves after inoculation with *T. hamatum* T382, which resulted in the suppression of subsequent infection by *B. cinerea* (Mathys *et al*., 2012).

Only a limited number of MAMP–PRR pairs have been characterized (Boller and Felix, 2009; Monaghan and Zipfel, 2012), including one example of a specific *Trichoderma* MAMP–PRR pair. A xylanase from *T. viride*, called the ethylene-inducing xylanase (Xyn2 or Eix), is a potent elicitor in tomato and tobacco (Rotblat *et al*., 2002) for which the corresponding receptor, EIX2, has been identified as a receptor-like protein with an extracellular LRR domain, a transmembrane domain and a short cytoplasmic tail without kinase domain (Ron and Avni, 2004). Another recently characterized MAMP–PRR pair possibly involved in the recognition of *Trichoderma* spp. by the plant is the *A. thaliana* lysine motif (LysM) receptor-like kinase CERK1 (chitin elicitor receptor-like kinase 1), which binds chitin (Iizasa *et al*., 2010; Petutschnig *et al*., 2010). It has been established that perception of chitin oligosaccharides through such LysM receptors contributes to disease resistance in *A. thaliana* (Wan *et al*., 2008). The recognition of chitin is also supported by our above-mentioned transcriptome study of the *A. thaliana–T. hamatum* T382 interaction (Mathys *et al*., 2012), indicating that the response to chitin is one of the most significantly induced biological processes in *A. thaliana* leaves in response to *T. hamatum* T382. Brotman *et al*. (2013) found the same GO process to be significantly enriched in *A. thaliana* roots, 24 h after inoculation with *T. asperelloides* T203. Hermosa *et al*. (2012) also proposed that chitin oligosaccharides are indirect inducers of MTI, as they can be released by the activity of both plant and *Trichoderma* chitinases. In addition, several proteomic and transcriptomic studies have shown that plant interactions with various *Trichoderma* spp. result in the induction of cytoplasmic NB-LRRs (Marra *et al*., 2006; Palmieri *et al*., 2012; Perazzolli *et al*., 2012; Shoresh and Harman, 2008), suggesting that *Trichoderma* spp., apart from increasing MTI, can also have an effect on the plant response by increasing ETI.

Plant responses induced by *Trichoderma* spp.: activation of signal transduction pathways

On MAMP recognition, mitogen-activated protein kinase (MAPK) cascades are activated as an early plant response (Boller and Felix, 2009). MAPK cascades convert extracellular stimuli into intracellular responses, amplifying, at the same time, the transducing signal via three reversibly phosphorylated kinases (Ichimura *et al*., 2002; Opdenakker *et al*., 2012; Rodriguez *et al*., 2010). Shoresh *et al*. (2006) demonstrated that a cucumber *MAPK* homologous to

A. thaliana MPK3 is activated by the inoculation of roots with *T. asperellum* T203. Moreover, induction of this *Trichoderma-induced protein kinase* (*TIPK*) appeared to be necessary for ISR against bacterial pathogens, whereas silencing of *TIPK* completely eliminated this protection (Shoresh *et al*., 2006). The homologous *A. thaliana MPK3* and a MAPK-kinase encoding gene (*MKK4*) were also induced in *A. thaliana* leaves after treatment of the roots with *T. hamatum* T382, resulting in reduced disease symptoms caused by *B. cinerea* (Mathys *et al*., 2012). Activation of MAPK cascades leads to the phosphorylation of substrate proteins, whose altered activities mediate a wide array of responses which are interconnected to other signalling molecules, such as plant hormones (Rodriguez *et al*., 2010).

Plant responses induced by *Trichoderma* spp.: plant hormone signalling

The action of plant hormones regulates the defence network that translates *Trichoderma* spp.-induced early signalling events into the activation of effective defence responses. Two main mechanisms are recognized: systemic acquired resistance (SAR) and ISR. SAR is traditionally defined as being triggered by pathogen infection, providing long-term systemic resistance to subsequent pathogen attack, which is correlated with the activation of pathogenesis-related (PR) proteins and requires the involvement of the signal molecule salicylic acid (SA) and subsequent mediation of the positive regulator protein NPR1 (Durrant and Dong, 2004). Early research on ISR in *A. thaliana* mainly focused on rhizobacteria-ISR (van Loon *et al*., 1998), which is thought to be regulated by ethylene (Et)- and jasmonic acid (JA)-mediated signalling pathways (Pieterse *et al*., 1998), with mediation of NPR1, but without induction of the SA-responsive genes *PR1*, *PR2* and *PR5* (Pieterse *et al*., 1996). However, this traditional view on ISR appears to be more complex. In recent years, it has become clear that an intensive interplay between hormone signalling pathways exists, the outcome of which determines the effectiveness of the immune response to a specific type of invader (Zamioudis and Pieterse, 2012).

Trichoderma spp. seem to be able to activate both the SA- and JA/Et-mediated signal transduction pathways, although the pattern varies depending on the experimental conditions and organisms involved (Contreras-Cornejo *et al*., 2011; Salas-Marina *et al*., 2011; Velazquez-Robledo *et al*., 2011). This may be an important trait for *B. cinerea* management, as resistance to this pathogen is largely dependent on complex overlapping signalling pathways, involving both SA- and JA/Et-mediated signal transduction pathways (Audenaert *et al*., 2002; Diaz *et al*., 2002; El Oirdi *et al*., 2011; Windram *et al*., 2012). Transcriptomic studies focusing on the plant response after inoculation with *Trichoderma* spp. are generally in line with this, although, depending on the study, more weight can be attributed to one of the two pathways. We recently investigated the response of *A. thaliana* leaves at the transcriptome level, 48 h after root inoculation with *T. hamatum* T382, referred to as the 'ISR-prime' phase, to indicate that pathogen infection had not yet occurred. GO analysis revealed a significant induction of the response to SA, whereas responses to JA and Et were not altered significantly (Mathys *et al*., 2012). We furthermore found a striking resemblance between the ISR-prime response and SAR response. Indeed, GO analysis revealed a strong induction of the biological process 'SAR' and 'regulation of SAR' during the ISR-prime phase, which was further highlighted by the induction of genes belonging to the SA pathway. This included, for example, the induced expression of *WRKY6*, *WRKY53*, *PR1*, *PR2* and *PR5*, and was also confirmed by the analysis of *A. thaliana* NahG, *npr1* and *sid2* mutants. Despite the similarities with the defence response induced by *B. cinerea*, the ISR-prime phase could be distinguished by the much more pronounced induction of the SA pathway, the production of SA via isochorismate instead of phenylalanine and the absence of involvement of the JA and Et pathways (Mathys *et al*., 2012). Moran-Diez *et al*. (2012) performed a transcriptome analysis of *A. thaliana* leaves, 24 h after incubation with *T. harzianum* T34 in liquid culture. A very limited number of differentially expressed genes could be detected, and genes involved in both SA and JA/Et signalling pathways were mainly down-regulated, although further analysis of marker genes for both pathways 48 h after incubation revealed an increased expression (Moran-Diez *et al*., 2012). These findings could suggest that *T. harzianum* T34 suppresses the initial plant defence response in order to allow root colonization, which is in contrast with another recently performed transcriptome analysis of *A. thaliana* roots in the interaction with *T. asperelloides* T203, which did not indicate major down-regulation of defence-related processes at the same time point in the roots (Brotman *et al*., 2013). This study revealed a more central role for JA, with the enhanced expression of several genes related to JA biosynthesis and JA signalling in the roots 24 h after *T. asperelloides* inoculation. For example, the authors reported the enhanced expression of the transcription factors WKRY18 and WRKY40, which stimulate JA signalling via suppression of JAZ repressors. In addition, GO analysis indicated the significant enrichment of JA biosynthesis.

A few transcriptomic studies have also been performed with other plants. Concerning the role of the main hormonal pathways in these studies, the transcriptome analysis of tomato leaves after root inoculation with *T. hamatum* T382 only revealed the induced expression of *PR5* (Alfano *et al*., 2007), whereas the transcriptome analysis of grapevine leaves after leaf treatment with *T. harzianum* T39 mainly pointed to the activation of Et metabolism (Perazzolli *et al*., 2012). Activated Et metabolism was also found in our very recently performed detailed transcriptome analysis of the tomato–*T. hamatum* T382 interaction, in addition to a clear up-regulation of many JA biosynthesis-related genes (Yang, 2013). The comparison of the tomato transcriptomic study with

that in *A. thaliana* (Mathys *et al*., 2012) enabled us to determine to what extent the ISR mechanisms of *T. hamatum* T382 can be extrapolated from one experimental system to another. Changing the model plant *A. thaliana* for the crop plant tomato revealed significant overlap in the ISR-prime mechanisms, as well as specificities for each interaction. For example, the JA biosynthesis pathway was found to be most prominently induced in tomato, whereas the induction of the SA signalling pathway appeared mainly in *A. thaliana*.

The involvement of JA, Et and SA in *Trichoderma* spp.–plant interactions is also often deduced from expression studies on marker genes linked to these signalling pathways. For example, up-regulation of *Lox1* (*lipoxygenase 1*), which encodes a lipoxygenase involved in JA synthesis, was observed after inoculation with *T. atroviride* IMI206040 in both the roots and leaves of *A. thaliana* (Salas-Marina *et al*., 2011). Expression studies with transgenic *A. thaliana* reporter plants portraying activity of the *Lox2* promoter showed the induction of this gene after inoculation with *T. virens* Tv.29-8 and *T. atroviride* IMI206040 (Contreras-Cornejo *et al*., 2011). A marker gene for JA/Et-mediated signalling in *A. thaliana*, *PDF1.2a* (encoding plant defensin 1.2; Thomma *et al*., 1998), was also up-regulated in both roots and leaves after *T. atroviride* treatment (Salas-Marina *et al*., 2011). The same study also showed increased expression of SA-inducible *PR* genes (*PR1* and *PR2*) after treatment with *T. atroviride*, both locally in roots and systemically in leaves. A similar induction of *PR1* by *T. virens* Tv29.8 was detected by Velazquez-Robledo *et al*. (2011) using transgenic *A. thaliana* reporter studies. The *Lox2* and *PR1* responses were absent in reporter plants co-cultivated with a mutant of *T. virens* which was unable to induce resistance against *B. cinerea*, suggesting that these genes are involved in resistance against *B. cinerea*, as the mutant colonized the root system as well as the wild-type strain (Velazquez-Robledo *et al*., 2011). In tomato, Tucci *et al*. (2011) detected the transcriptional activation of several *PR* genes in leaves after the addition of *T. harzianum* T22 and *T. atroviride* P1. For example, the PR1 family gene *PR1b1* (Tornero *et al*., 1997) and the PR4 family gene *PR-P2*, mainly induced by SA in tomato (Van Kan *et al*., 1995), showed induced gene expression after *Trichoderma* inoculation. However, Martínez-Medina *et al*. (2013) did not observe an increased expression of the SA-inducible gene *PR1a* in tomato leaves after treatment with *T. harzianum* T78. By contrast, the JA-responsive genes *PI II*, *MC* and *PS*, coding for proteinase inhibitor II, multicystatin and prosystemin, respectively, showed increased expression (Martínez-Medina *et al*., 2013). Yoshioka *et al*. (2012) reported a significant increase in expression of both the SA-inducible genes *PR1*, *PR2* and *PR5* and JA/Et-inducible genes, such as *PDF1.2a*, in *A. thaliana* leaves after root treatment with *T. asperellum* SKT-1. Remarkably, even the application of the cell-free culture filtrate of this strain led to an increased expression of the same marker genes (Yoshioka *et al*., 2012). Further evidence for the induction of JA and SA by *Trichoderma* spp. comes from metabolic studies. Levels of JA and SA were shown to accumulate in above-ground plant parts of cucumber and *A. thaliana* after the application of *T. asperellum* T34 or *T. virens* and *T. atroviride*, respectively (Contreras-Cornejo *et al*., 2011; Segarra *et al*., 2007). In cucumber, a proteomic study also revealed that *T. asperellum* T34 up-regulates the Et biosynthesis enzyme aminocyclopropane-1-carboxylate oxidase 1 (Segarra *et al*., 2007). The reported induction of *PR* gene expression described above is in agreement with proteomic analyses of bean and maize interacting with *T. atroviride* P1 (Marra *et al*., 2006) and *T. harzianum* T22 (Shoresh and Harman, 2008), respectively.

However, the impact of *Trichoderma* colonization on plant hormone signalling is not limited to the typical defence-related hormonal pathways of JA, Et and SA alone. For example, GO analysis also revealed the activation of the response to abscisic acid (ABA) in the interaction of *T. hamatum* T382 with either *A. thaliana* or tomato (Mathys *et al*., 2012; Yang *et al*., 2013), and Martínez-Medina *et al*. (2011) found that the ABA level in melon shoots was increased significantly after root treatment with *T. harzianum* T-78. Recently, the same authors also reported an up-regulation of the ABA-related marker gene *Le4*, encoding a desiccation protective protein, in tomato leaves after pre-inoculation of the roots with the T-78 strain (Martínez-Medina *et al*., 2013). ABA is an important regulator that balances responses to abiotic and biotic stresses in plants, but its exact role in plant–*Trichoderma* interactions is not yet clear. The same holds true for another class of hormones, the gibberellins (GA). GA can control the onset of JA- and SA-dependent defence responses through the regulation of DELLA protein degradation (Hermosa *et al*., 2012), but, so far, only a few papers have reported the involvement of the GA-associated pathway in *Trichoderma*-induced ISR. For example, the ISR phenotype was disrupted in the GA-impaired *A. thaliana* mutant *spy3*, despite treatment with *T. harzianum* T39 (Korolev *et al*., 2008), and an increase in GA levels in tomato roots was found after the application of *T. harzianum* OTPB3 to tomato seeds (Chowdappa *et al*., 2013).

Plant responses induced by *Trichoderma* spp.: production of secondary metabolites

Together with other plant defence strategies, the production of phytoalexins plays an important role in defence against *B. cinerea*. The phenylpropanoid pathway, with phenylalanine ammonium lyase (PAL) as the first enzyme, is a major source for the production of antimicrobial phenolic compounds, as well as SA precursors (Huang *et al*., 2010; Mauch-Mani and Slusarenko, 1996). Brotman *et al*. (2013) found an increased expression of several genes involved in the phenylpropanoid pathway, such as PAL1, PAL2 and 4CL, in *A. thaliana* roots after inoculation with *T. asperelloides* T203, but Martínez-Medina *et al*. (2013) did not observe an increased expression of PAL in tomato leaves after colonization

with *T. harzianum* T78. Calderon *et al*. (1993) demonstrated that cell suspension cultures of grapevine treated with an elicitor from *T. viride* produced increased amounts of resveratrol, a phytoalexin with growth inhibitory activity towards *B. cinerea* (Ali *et al*., 2003). Later studies also proved that phytoalexins from other plant species can effectively inhibit the proliferation of *B. cinerea* (Chassot *et al*., 2008; Denby *et al*., 2004; Ferrari *et al*., 2003). More extensive phytoalexin research has been performed in the model plant *A. thaliana*. The last step in the biosynthesis of its most abundant phytoalexin, camalexin, is catalysed by a cytochrome P-450 enzyme CYP71B15 (or PAD3). Mutants deficient in camalexin production, such as *pad3* (*phytoalexin deficient 3*), display enhanced susceptibility to *B. cinerea* (Ferrari *et al*., 2003). Interestingly, *PAD3* expression was found to be up-regulated in both roots and leaves after treatment of *A. thaliana* with *T. atroviride* (Salas-Marina *et al*., 2011). Similarly, *A. thaliana* seedlings colonized with wild-type *T. virens* accumulated higher levels of camalexin than axenically grown seedlings or plants co-cultivated with a *T. virens* mutant unable to trigger ISR (Velazquez-Robledo *et al*., 2011). Such induction of camalexin levels in *A. thaliana* seedlings was confirmed in another study using both *T. virens* and *T. atroviride* (Contreras-Cornejo *et al*., 2011). In addition, the authors observed that *T. virens* produces indole-3-carboxaldehyde, a possible precursor of camalexin (Devys and Barbier, 1991; Zook and Hammerschmidt, 1997). Moreover, this compound induces camalexin accumulation when externally applied to *A. thaliana* seedlings, suggesting that the plant might use this compound for camalexin production by either direct or indirect means (Contreras-Cornejo *et al*., 2011). Furthermore, the same authors observed induced levels of anthocyanin, which corresponds to our recent observations of a very pronounced induction of anthocyanin biosynthesis after inoculation of *A. thaliana* roots with *T. hamatum* T382 via both transcriptomic analysis and the chemical quantification of anthocyanin levels (Mathys *et al*., 2012). Moreover, we showed that *T. hamatum* T382 was unable to induce ISR in *A. thaliana* mutant plants affected in different parts of the phenylpropanoid pathway. However, we did not observe a significant up-regulation of the genes involved in camalexin biosynthesis in the ISR-prime phase. The induction of the phenylpropanoid pathway instead of the camalexin pathway as the main source of secondary metabolites in the ISR-prime phase induced by *T. hamatum* T382 was proposed to be one of the characteristic differences to distinguish the ISR-prime phase from the plant response to *B. cinerea* (Mathys *et al*., 2012). In addition, we found an apparent overlap in the induction of the phenylpropanoid pathway in the ISR-prime phase in both *A. thaliana* and tomato (Yang, 2013).

In addition to the involvement of the phenylpropanoid pathway, the expression of genes encoding lipid transfer proteins (LTPs) (PR-14) is also often reported to be influenced by *Trichoderma* colonization. Many biological roles have been suggested for LTPs, including a role in plant defence. Antimicrobial activity has been demonstrated for various LTPs against several fungi and bacteria, probably resulting from their capacity to interact with biological membranes, which may lead to membrane permeabilization (reviewed by Sels *et al*., 2008). Proteome analysis revealed that the spraying of grapevine leaves with *T. harzianum* T39 increased the abundance of an LTP in the leaves (Palmieri *et al*., 2012), and various transcriptome studies have also reported an altered expression of LTPs. For example, Moran-Diez *et al*. (2009) compared the differential expression of *A. thaliana* genes during interaction with either a wild-type *T. harzianum* T34 or an endopolygalacturonase-silenced strain. One of the 10 genes that differed significantly in expression between both interactions was an LTP, with a reduced expression level in the presence of the silenced strain. Multiple LTPs have been reported to be up-regulated after root colonization with various *Trichoderma* strains in *A. thaliana*, pepper and cocoa (Bae *et al*., 2011; Bailey *et al*., 2006; Moran-Diez *et al*., 2012), as well as in the ISR-boost phase of the *A. thaliana–B. cinerea–T. hamatum* T382 interaction (Mathys *et al*., 2012). The possible importance of LTPs in defence against *B. cinerea* has been demonstrated, for example, by Chassot *et al*. (2007), who showed that endogenous overexpression of three LTP-like genes in *A. thaliana* resulted in enhanced tolerance to this pathogen.

Plant responses induced by *Trichoderma* spp.: control of ROS damage

Although phytoalexins can have a direct inhibitory effect on *B. cinerea*, inoculation with *Trichoderma* spp. can also induce plant responses that are more indirectly involved in the control of *B. cinerea* disease, by interfering with the pathogen's infection strategy. The oxidative burst, characterized by the rapid generation of ROS and the accumulation of H_2O_2 (Lamb and Dixon, 1997), belongs to the early events of both resistant and susceptible plant responses to *B. cinerea* (Govrin and Levine, 2000). It was demonstrated that *B. cinerea* actively contributes to the generation of an oxidative burst during the plant–pathogen interaction, by both direct generation of ROS and the secretion of enzymes and metabolites that influence the oxidative burst in the plant (Schouten *et al*., 2002; Temme and Tudzynski, 2009). The production of ROS and concomitant host cell death occurring early after infection by *B. cinerea* are indicative of a successful infection, as *B. cinerea*, a necrotroph, benefits from dead tissue (Govrin *et al*., 2006). In addition, it has been established that, during plant–pathogen interactions, ROS can also act as signalling molecules, inducing a MAPK cascade and eliciting a defence response (Hancock *et al*., 2002). In this respect, our transcriptome analysis of the interaction between *T. hamatum* T382 and *A. thaliana* revealed a pronounced induction of ROS-inducible genes in the leaves, supposedly followed by the induction of a MAPK cascade (Mathys *et al*., 2012).

Because of the dual functions of ROS, controlled generation is of tremendous importance for the outcome of *B. cinerea* infection. Positive interference of *Trichoderma* spp. with ROS production, e.g. through an increase in the plant's ROS-scavenging abilities at future infection sites, can potentially reduce the damaging levels of ROS in leaves, thus limiting *B. cinerea* infection. In this context, our transcriptome analysis of the ISR-prime phase in the *A. thaliana–T. hamatum* T382 interaction showed the induction of multiple peroxidases, glutathione-reductases, glutathione-*S*-transferases and other detoxifying enzymes in *A. thaliana* leaves (Mathys *et al*., 2012); comparable results were also obtained in our tomato–*T. hamatum* T382 ISR-prime study (Yang, 2013), as well as by Perazzolli *et al*. (2012) for grapevine leaves after leaf treatment with *T. harzianum* T39. Similarly, in another study, the expression level of a peroxidase-encoding gene was demonstrated to increase in both roots and leaves of *A. thaliana* after inoculation with *T. atroviride* (Salas-Marina *et al*., 2011). This was confirmed by a proteomic study on maize seedlings root inoculated with *T. harzianum*, which demonstrated increased levels of superoxide dismutase, peroxidase, glutathione-reductase, glutathione-*S*-transferase and other ROS-scavenging enzymes in leaves (Shoresh and Harman, 2010). Recently, Brotman *et al*. (2013) also reported the increased expression of genes encoding antioxidant enzymes in roots of both *A. thaliana* and cucumber after inoculation with *T. asperelloides* T203. For example, the *MDAR* gene, encoding monodehydroascorbate reductase, was significantly up-regulated in both plants.

Responses induced by *B. cinerea* in *Trichoderma*-triggered plants

The activation of plant signalling pathways and the reprogramming of plant gene expression by *Trichoderma* spp., as described in the previous section, are responsible for the establishment of a unique physiological situation, called the 'primed' state of the plant (Conrath, 2009; Shoresh *et al*., 2010). This primed condition can also be achieved after the colonization of the roots by other beneficial microbes, infection by a pathogen, treatment with various chemicals or wounding (Conrath, 2009). Primed plants respond more rapidly and/or more strongly through the activation of defence responses when subsequently challenged by microbial pathogens, herbivorous insects or abiotic stresses (Conrath, 2009, 2011). To demonstrate this change in (defence) response on pathogen challenge in primed plants, an examination of the three-player interaction (BCO–plant–pathogen) is necessary. Evidence has been provided for a priming effect induced by *Trichoderma* spp. against diverse types of pathogens (Brotman *et al*., 2012; Gallou *et al*., 2009; Perazzolli *et al*., 2011; Shoresh *et al*., 2005; Yedidia *et al*., 2003), but data are limited and mostly fragmentary. Moreover, the different studies applied different experimental set-ups, making comparison of the data obtained difficult. With respect to *B. cinerea*, to our knowledge, we were the first to report a transcriptome analysis on such a three-player interaction, in which the gene expression profiles induced by inoculation with *B. cinerea* in *A. thaliana* plants, pre-inoculated with *T. hamatum* T382, were compared with those in *B. cinerea* only-infected plants (Mathys *et al*., 2012). We termed this phase the 'ISR-boost phase' to distinguish it from the ISR-prime phase before *B. cinerea* infection. Pre-inoculation with *T. hamatum* T382 appeared to prime the plant to respond more quickly to *B. cinerea* infection, as GO analysis revealed a transient activation of the JA biosynthesis process, which was not observed in plants without *T. hamatum* T382 pre-inoculation. The responses to JA and wounding, both linked to the JA pathway, were also reinforced in the *T. hamatum* T382-treated plants. Furthermore, genes involved in the biosynthesis of secondary metabolites, such as anthocyanins, flavonoids and galactolipids, were induced, which was not observed in *B. cinerea* only-infected plants. The former was also confirmed via chemical analysis of anthocyanin content (Mathys *et al*., 2012). However, a clear moderation of transcriptional changes induced by *B. cinerea* (including GO terms dealing with defence responses, hormone pathways and camalexin production) was also observed, possibly resulting from the priming effect and subsequent inhibition of *B. cinerea* proliferation. In addition, an impaired ISR phenotype against *B. cinerea* was observed in both JA and SA mutants of *A. thaliana*, but not in Et mutants (Mathys *et al*., 2012).

More evidence on the involvement of JA- and Et-mediated pathways in *Trichoderma* spp.-induced ISR against *B. cinerea* comes from a phenotypic study by Korolev *et al*. (2008) on a series of *A. thaliana* mutants with impaired JA/Et-mediated responses. In contrast with their wild-type plants, none of the mutants developed ISR against *B. cinerea* in response to the treatment of roots with *T. harzianum* T39 (Korolev *et al*., 2008). Interestingly, four mutants impaired in SA-mediated signalling were also analysed, but the ability of *T. harzianum* T39 to induce ISR against *B. cinerea* was not affected in these plants (Korolev *et al*., 2008). The results obtained with tomato mutants confirm the role of the main plant hormones in the ISR response against *B. cinerea* (Martínez-Medina *et al*., 2013). Root colonization by *T. harzianum* T78 significantly reduced *B. cinerea* infection in tomato wild-types, but not in mutants impaired in the biosynthesis of JA or in the accumulation of SA or ABA, thus indicating a role for these plant hormones in the ISR response. Further expression analysis also revealed that the JA-responsive genes *PI II*, *MC* and *PS*, which were already slightly induced in the ISR-prime phase, were induced to much higher levels in the ISR-boost phase, thus indicating the importance of the priming of JA-dependent defence responses in the *T. harzianum*-induced biocontrol against *B. cinerea* in tomato (Martínez-Medina *et al*., 2013). In addition, using tomato mutants, we recently observed the need for a functional JA and phenylpropanoid biosynthesis pathway for the ISR induced by *T. hamatum* T382 against *B. cinerea* (Yang *et al*.,

2013). A small transcriptional study in tomato performed by Tucci *et al*. (2011) demonstrated that the expression of two JA/Et-responsive genes was induced and maintained at a higher level during *B. cinerea* infection in *Trichoderma*-treated versus untreated plants. This priming effect generally correlated with increased pathogen tolerance, although the authors observed that this response was dependent on the plant genotype and *Trichoderma* strains used. The opposite was true for two SA-responsive *PR* genes (of PR family 1 and 2), for which the expression in tomato plants infected by *B. cinerea* was lower in *T. harzianum*-treated relative to untreated plants. However, Marra *et al*. (2006) observed enhanced induction of a thaumatin-like protein (PR5b), which is also SA responsive, in a proteomic analysis of the tripartite interaction *T. atroviride*–bean–*B. cinerea*.

CONCLUSIONS AND PERSPECTIVES

Considering the potential direct effects of *Trichoderma* spp. on *B. cinerea*, as well as their ability to induce systemic resistance in the plant, they are very well suited to complement the current measures for the control of *B. cinerea* disease. Different mechanisms regarding the direct inhibition of *B. cinerea* by *Trichoderma* spp. have been described, often probably acting in combination. In the case of ISR against *B. cinerea*, the specific pathways involved also seem to vary according to the *Trichoderma* species under study. Furthermore, it has been shown that the plant genotype also plays an important role in the ISR-inducing ability of a *Trichoderma* strain (Korolev *et al*., 2008; Tucci *et al*., 2011; Yang, 2013). Moreover, the concentration of the *Trichoderma* spp.

Table 1 Overview of transcriptomic studies performed on bipartite and tripartite plant interactions with *Trichoderma* spp. and *Botrytis cinerea*.

Plant species	Plant tissue	*Trichoderma* species	Time point(s) of analysis	Remarks	Reference
Bipartite interactions: *Trichoderma* spp.–plant					
Arabidopsis thaliana	Aerial parts	*T. harzianum* T34	1 dpTi	Microarray	Moran-Diez *et al*. (2012)
Arabidopsis thaliana	Aerial parts	*T. hamatum* T382	2 dpTi	Microarray	Mathys *et al*. (2012)
Arabidopsis thaliana	Roots	*T. asperelloides* T203	1 dpTi	Microarray, salt stress applied	Brotman *et al*. (2013)
Solanum lycopersicum	Leaves	*T. hamatum* T382	5 wpTi	Microarray; very low number of DE genes	Alfano *et al*. (2007)
Solanum lycopersicum	Leaves	*T. hamatum* T382	2 dpTi	Microarray	Yang (2013)
Vitis vinifera	Leaves	*T. harzianum* T39	1 dpTi	RNA-seq, interaction with *Plasmopara viticola*; T39 applied to leaves	Perazzolli *et al*. (2012)
Bipartite interactions: *B. cinerea*–plant					
Arabidopsis thaliana	Aerial parts	–	0, 24, 36, 60 hpBi	Microarray, comparison with mutant plants	AbuQamar *et al*. (2006)
Arabidopsis thaliana	Leaves	–	18, 48 hpBi	Microarray, comparison with elicitor treatment	Ferrari *et al*. (2007)
Arabidopsis thaliana	Leaves	–	14 hpBi	Microarray, comparison with mutant plants	Birkenbihl *et al*. (2012)
Arabidopsis thaliana	Systemic leaves	–	1, 2 dpBi	Microarray	Mathys *et al*. (2012)
Arabidopsis thaliana	Leaves/leaf discs	–	12, 24, 48 hpBi	Microarray, also spatial analysis of leaf response	Mulema and Denby (2012)
Arabidopsis thaliana	Detached leaves	–	Every 2 h up to 48 hpBi	High-resolution microarray	Windram *et al*. (2012)
Lactuca sativa	Leaves	–	12, 24, 48 hpBi (local leaves) 48, 72, 96 hpBi (systemic leaves)	RNA-seq	De Cremer *et al*. (2013)
Solanum lycopersicum	Detached leaves	–	0, 8 hpBi	Microarray, comparison with mutant plants	Asselbergh *et al*. (2007)
Solanum lycopersicum	Fruits	–	1 dpBi	Microarray, comparison of ripening stages	Cantu *et al*. (2009a)
Solanum lycopersicum	Fruits	–	1 dpBi	Transcriptome analysis of hormone-related genes	Blanco-Ulate *et al*. (2013)
Tripartite interactions: *Trichoderma* spp.–*B. cinerea*–plant					
Arabidopsis thaliana	Leaves	*T. hamatum* T382	2 dpTi (ISR-prime phase) 1, 2 dpBi (ISR boost phase)	Microarray; systemic leaves used in boost samples	Mathys *et al*. (2012)

dpBi, days post-*B. cinerea* inoculation; dpTi, days post-*Trichoderma* spp. inoculation; hpBi, days post-*B. cinerea* inoculation; wpTi, weeks post-*Trichoderma* spp. inoculation; ISR, induced systemic resistance; DE, differentially expressed.

inoculum, the developmental stage of the plant and the timing of the interaction have also been shown to affect the *Trichoderma* spp.–plant interaction (Contreras-Cornejo *et al*., 2011; Hermosa *et al*., 2012). Above all, the success of *Trichoderma* spp. as BCOs depends strongly on the challenging pathogen, which was focused in this review on *B. cinerea*, an important pathogen of current agricultural crops with one of the broadest host spectra.

So far, large-scale transcriptional profiling of the plant response following infection with *B. cinerea* has mainly been performed in *A. thaliana*, with a few studies carried out in tomato and recently also in lettuce (Table 1). Similarly, transcriptional profiling of the *Trichoderma*–plant interaction has only been reported in a few studies, focusing on different *Trichoderma* strains and plant species (Table 1). Concerning tripartite interactions, however, genome-wide analyses that could drastically advance our understanding of ISR mechanisms remain extremely scarce to date. For *B. cinerea*, our recent transcriptomic study on the tripartite interaction in the *A. thaliana–T. hamatum* T382 experimental system is, to our knowledge, the only one published so far (Mathys *et al*., 2012; Table 1). With the emergence of next-generation sequencing techniques and the increasing amount of available sequenced genomes of all players in the tripartite interactions, however, this is expected to change in the years ahead, which will undoubtedly result in a great leap forward in our understanding of tripartite *Trichoderma–B. cinerea* biocontrol interactions.

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